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Vangsgaard, Anna Katrine; Mauricio Iglesias, Miguel; Valverde Perez, Borja; Gernaey, Krist; Sin, Gürkan

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pH variation and influence in an autotrophic nitrogen removing biofilm system using an efficient numerical solution strategy

Anna Katrine Vangsgaard, Miguel Mauricio-Iglesias, Borja Valverde-Pérez, Krist V. Gernaey and Gürkan Sin

ABSTRACT

A pH simulator consisting of an efficient numerical solver of a system of nine nonlinear equations was constructed and implemented in the modeling software MATLAB. The pH simulator was integrated in a granular biofilm model and used to simulate the pH profiles within granules performing the nitrification-anammox process for a range of operating points. The simulation results showed that pH profiles were consistently increasing with increasing depth into the granule, since the proton producing aerobic ammonium oxidizers (AOB) were located close to the granule surface. Despite this pH profile, more NH_3 was available for AOB than for anaerobic ammonium oxidizers (AnAOB), located in the center of the granules. However, operating at a higher oxygen loading resulted in steeper changes in pH over the depth of the granule and caused the NH_3 concentration profile to increase from the granule surface towards the center. The initial value of the background charge and influent bicarbonate concentration were found to greatly influence the simulation result and should be accurately measured. Since the change in pH over the depth of the biofilm was relatively small, the activity potential of the microbial groups affected by the pH did not change more than 5% over the depth of the granules.

Key words | anammox, autotrophic nitrogen removal, biofilm, granules, modeling, pH

Anna Katrine Vangsgaard (corresponding author)
Miguel Mauricio-Iglesias
Borja Valverde-Pérez
Gürkan Sin
 Computer Aided Process Engineering Center
 (CAPEC),
 Department of Chemical and Biochemical
 Engineering,
 Technical University of Denmark,
 Building 229,
 Søltofts Plads,
 2800 Lyngby,
 Denmark
 E-mail: akv@kt.dtu.dk

Krist V. Gernaey
 Center for Process Engineering and Technology
 (PROCESS),
 Department of Chemical and Biochemical
 Engineering,
 Technical University of Denmark,
 Building 229, Søltofts Plads,
 2800 Lyngby,
 Denmark

INTRODUCTION

Several studies have investigated complete autotrophic nitrogen removal, which combines partial nitrification with anaerobic ammonium oxidation (anammox), as an energy and cost efficient alternative treatment method for wastewaters containing high concentrations of nitrogen and low concentrations of readily biodegradable organic matter (Strous *et al.* 1998). A one-stage treatment can be obtained in a biofilm reactor where both aerobic and anaerobic environments are created within the vicinity of each other, e.g. in granular sludge. Due to their spatial stratification granules can contain both aerobic ammonium oxidizing bacteria (AOB) and anaerobic ammonium oxidizing bacteria (AnAOB) performing the nitrification and anammox process, respectively. Among the operating conditions in the reactor, pH has a major impact on the nitrification and on the anammox process, since it has an impact on: (1) the compound speciation,

and thus on the substrate concentration; and, (2) the structure and stability of the bacterial cell wall and membrane. In addition, pH varies as a consequence of the activity of these microbial groups, due to their production or consumption of protons during their metabolism (Figure 1). One-stage nitrification-anammox is a novel and promising treatment technology, yet scale-up and stable full-scale operation has proved challenging (Joss *et al.* 2011), and further investigations on limiting factors and bottlenecks of the process should be conducted (van Hulle *et al.* 2010). The objective of this study is to improve the process understanding by elucidating the close relation between microbial activity and pH in a stratified biofilm structure by means of numerical model simulations. To reach this objective, an efficient pH calculation procedure including a pH model and numerical solution strategy was developed.

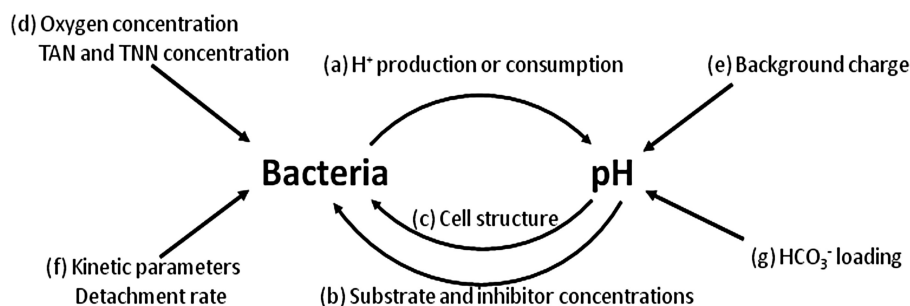


Figure 1 | Interactions between bacterial activity, pH and reactor environment. (a) The pH profile indicates where H^+ production/consumption takes place, (d) dictates the location of the bacterial groups. TAN + TNN in combination with pH (b) determines the substrate and inhibitor concentrations which affect bacteria location, (c) determines where in the biofilm growth conditions are the best, (e) & (g) affect the absolute value and shape of the pH profile, and (f) have a high impact on biomass activity and on the inert fraction of the granule.

MATERIALS AND METHODS

Model description

The model described a continuously run granular sludge reactor, and consisted of mass balances for soluble and particulate compounds within the biofilm and in the bulk liquid. One granule size was considered in the model. For model equations, parameter values, and further details, we refer to the model published in Vangsgaard *et al.* (2012). Just as in that model, aeration was included in the bulk liquid mass balance for oxygen. On the contrary, no stripping was considered for any of the other gaseous compounds (CO_2 or N_2).

The speciation of the true substrates has been, and still is, a point of discussion (Sin *et al.* 2008; Jin *et al.* 2012), with Anthonisen *et al.* (1976) being among the first to propose that NH_3 , rather than NH_4^+ or total ammonium nitrogen (TAN), is the true substrate for AOB. This has since been extended to AnAOB substrates with van Hulle (2005) showing that the unionized species were the true substrates. In this work, the unionized forms of the compounds were assumed to be the true substrates for all of the autotrophic microbial groups, i.e. AOB, nitrite oxidizing bacteria (NOB), and AnAOB. Thus AOB use NH_3 and are product inhibited by free nitrous acid (HNO_2), rather than by NO_2^- or total nitrite nitrogen (TNN), NOB grow on HNO_2 , and AnAOB utilize NH_3 and HNO_2 . The pH therefore has a significant impact on the substrate and inhibitor concentrations and availability (Figure 1). Additionally, pH affects the structure and permeability of the bacterial cell membrane and the energy required for maintenance of the internal cell proton concentration. To account for this, a bell-shaped function around the optimal pH value

(Henze *et al.* 1995) was included in the growth rates of AOB, AnAOB, and NOB with the parameter values obtained by van Hulle *et al.* (2007) and used by Ganigue *et al.* (2010).

pH calculation and numerical solution

The procedure of the determination of pH was carried out through the solution of a system of equations, consisting of three mass balances of TAN, TNN, and total inorganic carbon (TIC) (Equations (1a)–(1c)), five acid-base equilibrium conditions (Equations (2a)–(2e)), and a global charge balance (Equation (3)). In the global charge balance, the background charge (Z^+) represents the net charge that participates neither in acid/base equilibria nor in the biological conversions. A value of 10 charge-mol/ m^3 was used in all simulations. As an illustration, addition of NaCl does not contribute to Z^+ since its net charge is zero (the anion Cl^- and the cation Na^+ compensate each other) whereas addition of HCl would decrease the background charge (corresponding to the contribution of the anion Cl^- , which is completely dissociated). The resulting system of nine nonlinear equations was solved by a multidimensional Newton-Raphson method adapted from Luff *et al.* (2001).

$$0 = TAN - (NH_4^+ + NH_3) \quad (1a)$$

$$0 = TNN - (HNO_2 + NO_2^-) \quad (1b)$$

$$0 = TIC - (CO_2 + HCO_3^- + CO_3^{2-}) \quad (1c)$$

$$0 = K_w - OH^- \cdot H^+ \quad (2a)$$

$$0 = K_{e,NH_4} \cdot NH_4^+ - NH_3 \cdot H^+ \quad (2b)$$

$$0 = K_{e,HNO_2} \cdot HNO_2 - NO_2^- \cdot H^+ \quad (2c)$$

$$0 = K_{e,CO_2} \cdot CO_2 - HCO_3^- \cdot H^+ \quad (2d)$$

$$0 = K_{e,HCO_3} \cdot HCO_3^- - CO_3^{2-} \cdot H^+ \quad (2d)$$

$$0 = Z^+ - NO_3^- - HCO_3^- - 2 \cdot CO_3^{2-} - NO_2^- - OH^- + NH_4^+ + H^+ \quad (3)$$

The system of partial differential equations (PDEs) (the compound mass balances) was discretized in space into 100 nodes, each accounting for a given control volume, which resulted in a system of ordinary differential equations (ODEs). The numerical pH solver along with the model was implemented and solved during dynamic simulations in the software MATLAB R2009b (The MathWorks, Natick, MA). The built-in 'ode15s' solver based on numerical differentiation formulas was used to solve the ODEs. It was assumed that the establishment of the acid-base equilibria was immediate compared to the diffusion and production/consumption of compounds. As a consequence, a new distribution of the chemical species was calculated for every integration time step of the equation system.

However, it was quickly found out that the determination of pH through numerical solution of Equations (1)–(3) was computationally heavy and prone to numerical errors in some implementations, since the Newton-Raphson numerical method was not convergent for every initial guess. To overcome these problems and to obtain an efficient solution strategy, pH was determined off-line (still through the Newton-Raphson method), prior to simulation, for the complete expected range of TAN, TNN, TIC and NO_3^- concentrations. A lookup table was constructed from

these results using the Matlab command 'TriScatteredInterp' and then used to interpolate the value of pH during integration of the ODEs in dynamic simulations. The error of interpolation was estimated by evaluating the difference between the Newton-Raphson method solution and the interpolation. This was done for 10,000 points which were different from the ones used to build the interpolator. The expected root mean squared interpolation error was 0.014 pH units and the variance was 3.87e-4 (pH unit squared) for the range pH = 3 to pH = 9. If the range of pH was restricted to the range 6.5–8.5, the expected root mean squared interpolation error became 7.89e-5 and the variance 6.09e-9. This error was considered negligible.

For all simulations the initial conditions were the steady state result of a simulation using a constant pH value. To obtain a steady state solution, the dynamic model was solved for a long enough time (in this case 10,000 d) and the final values were recorded as steady state results.

Description of scenarios

Four scenarios comprising four different operating points were simulated and evaluated (see Table 1). The scenarios were based on a 4 L cylindrical lab-scale reactor with a diameter of 16 cm, operated for autotrophic nitrogen removal. The operating temperature was 25 °C, and the vessel was mechanically mixed by a Rushton impeller operated at 80 rpm and by bubble aeration. The solids concentration was approximately 3.14 g VSS/L. The average thickness of the external mass transfer boundary layer (L_B) was estimated to be 64 µm, by a semi-empirical correlation for stirred reactors with aeration (Nicollella et al. 1998).

In scenario 1, an oxygen mass transfer coefficient ($k_L a$) determined through clean water tests was used (43 d⁻¹), the volumetric N loading was 0.2 g TAN-N/L/d, and the bicarbonate loading was 1.22 g HCO_3^- /L/d, corresponding to a

Table 1 | Description of scenarios of autotrophic nitrogen removal system to study pH effects

Operation variable	Scenario 1–Mimicking lab-scale reactor	Scenario 2–Effect of increased aeration rate (double)	Scenario 3–Effect of smaller granule size	Scenario 4–Effect higher loadings
N loading	0.2 g L ⁻¹ d ⁻¹	0.2 g L ⁻¹ d ⁻¹	0.2 g L ⁻¹ d ⁻¹	0.65 g L ⁻¹ d ⁻¹
HRT	1 d	1 d	1 d	1 d
$k_L a$	43 d ⁻¹	86 d ⁻¹	43 d ⁻¹	140 d ⁻¹
Granule size (diameter)	2 mm	2 mm	0.5 mm	2 mm
WWT type	Low digester effluent strength	Low digester effluent strength	Low digester effluent strength	High digester effluent strength

molar ratio of 1:1.43 TAN-N:HCO₃⁻. In scenario 2, the effect of oxygen supply was investigated by doubling the value of the mass transfer coefficient ($k_L a$). In scenario 3, the effect of granule size was investigated by decreasing the granule diameter from 2 to 0.5 mm. In the model, the granules were considered identical, i.e. only one size class was included. Finally, the effect of high influent loading for a given amount of biomass was investigated in scenario 4. In the latter scenario, the increased loading of nitrogen implied increasing the supply of bicarbonate to 4.27 g HCO₃⁻/L/d and the oxygen supply to $k_L a = 140 \text{ d}^{-1}$ (see Table 1).

RESULTS AND DISCUSSION

From the modeling results it was found that the initial value of the background charge had a significant impact on the pH due to its impact on the solution of the system of equations (Equations (1)–(3)). Both the value of the pH and the shape of the pH profile were affected by this variable. The higher the background charge, the higher the pH and the flatter the pH profile. The background charge changes with composition and strength of wastewater, and estimation of this prior to pH calculation should therefore preferably be conducted. Unfortunately, the background charge is very difficult to estimate for a biofilm system for two reasons: the actual detailed composition of the biofilm

is not known (or not considered in the model, e.g. ions, along with the extracellular polymeric substances making up the biofilm matrix are not considered); and secondly, there is no reason why the background charge should be constant through the biofilm thickness.

Since background charge values are rarely reported and vary from system to system, the approach has been to use a value of Z^+ which would give a pH close to 7.2 in the bulk of the reactor, given the concentrations predicted for a constant pH. This resulted in the Z^+ value being 10 charge-mol/m³. The simulations with such a value of charge and with the given bicarbonate concentrations lead to different but relatively close values of pH in the bulk (from ~7.2 in scenario 4 to 7.6 in scenarios 1 and 3, as it can be seen below).

The results shown in the Figures 2–5 were steady state conditions obtained after simulating 10,000 d of operation.

Scenario 1. Interpretation of the results

The pH decreased from the center to the surface of the biofilm, where AOB were present at the biofilm/liquid interface (Figure 2(c)). The combination of the TAN concentration profile and the pH profile yielded a NH₃ profile, which despite the shape of the pH profile, showed that less NH₃ was available as substrate for AnAOB located a bit further inside the granule (Figure 2(c)) than there was for the

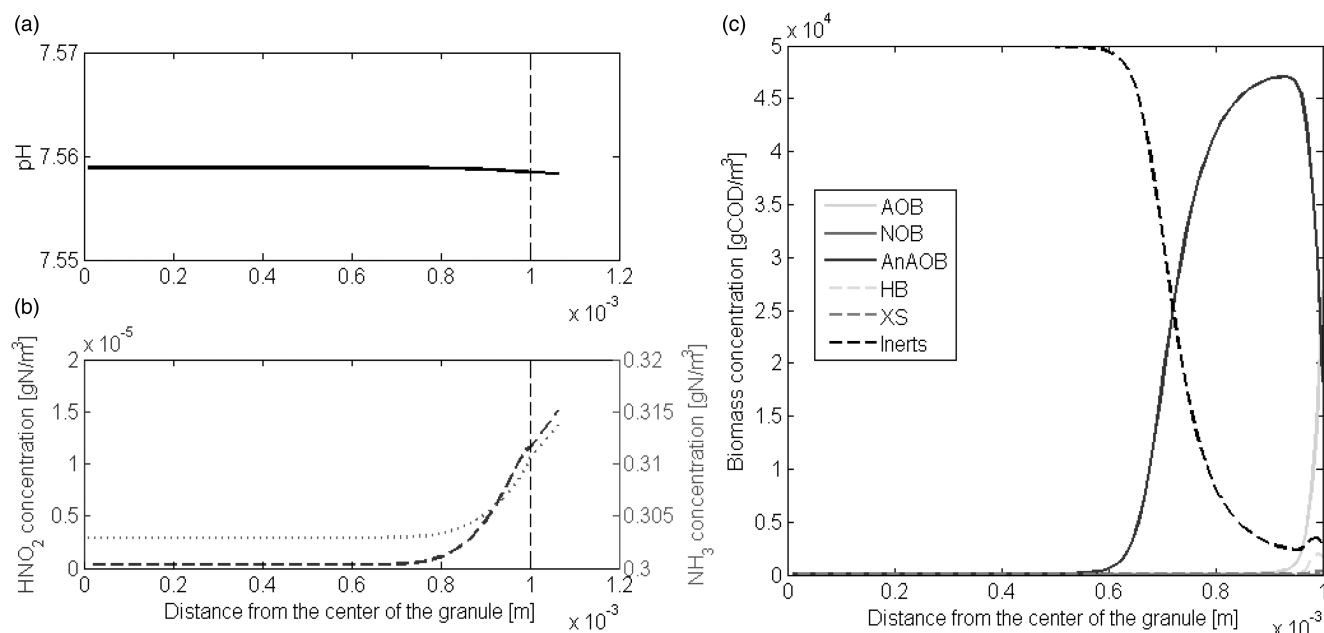


Figure 2 | (a) pH profile with biofilm depth, (b) NH₃ and HNO₂ profiles biofilm with biofilm depth, and (c) biomass composition inside the granular biofilm in scenario 1 simulated with pH effect on microbial growth. The vertical dashed line in (a) and (b) indicates the biofilm/liquid interface.

AOB located at the surface. Both the pH profile and the HNO_2 profile, which also showed a decreasing trend from the surface towards the center of the granule, were the opposite compared to observations made by Park *et al.* (2010). The difference can be found in the consumption of TNN, which in this study is taking place inside the granule at the location of the AnAOB (Figure 2(c)). AOB consumed significantly more alkalinity than the AnAOB, which was evident from the pH profile showing that the greatest change was happening in the vicinity of the biofilm/liquid interface where the AOB were present (illustrating process (a) in Figure 1). The microbial composition in this scenario was similar to investigations made without inclusion of the pH effect (Volcke *et al.* 2010; Vangsgaard *et al.* 2012), which is reasonable considering the relatively small change in pH value over the depth of the biofilm. The total nitrogen removal efficiency of the granular reactor was 86.4%.

Scenarios 2–4. Effect of operating conditions

Simulation of scenario 2 resulted in a similar pH profile as obtained in scenario 1, with a lower pH closer to the biofilm/liquid interface and increasing towards the granule center (Figure 3(a)). However, the shape of the pH profile was very different with the pH changing over almost the entire biofilm depth, instead of just close to the biofilm/liquid interface, and the slope of the pH profile was much

steeper. The effect of the relatively high change in pH resulted in an NH_3 concentration profile showing an increasing trend from the granule surface towards the center of the granule. The AnAOB were located from a couple of hundred micrometers below the surface of the biofilm to the center, where there was a tradeoff between availability of NH_3 and HNO_2 and low oxygen concentrations (Figure 3(c)). It could also be observed that NOB growth appeared close to the biofilm/liquid interface under these conditions. The more oxygen supplied thus caused nitrification to be the dominating process and the nitrogen removal efficiency was therefore only 27.2%, while the TAN removal efficiency was 83.5%.

At smaller granule sizes, similar results as in scenario 1 were observed, showing a relatively small difference in pH value over the depth of the biofilm, but with the difference that the pH profile was smoother with on the one hand a reduced slope, and on the other hand a gradient that was penetrating deeper in the granule. The NH_3 concentration did therefore not change much over the depth as a result, and the HNO_2 was mainly a function of the TNN concentration profile. The HNO_2 penetrated all the way through the granule, albeit in low concentrations in the center of the granule (Figure 4(b)). As a result the granules contained higher amounts of AnAOB, and the inert core took up less space (Figure 4(c)) than in scenario 1. Despite the change in microbial composition, the efficiency of the total nitrogen removal remained similar to scenario 1.

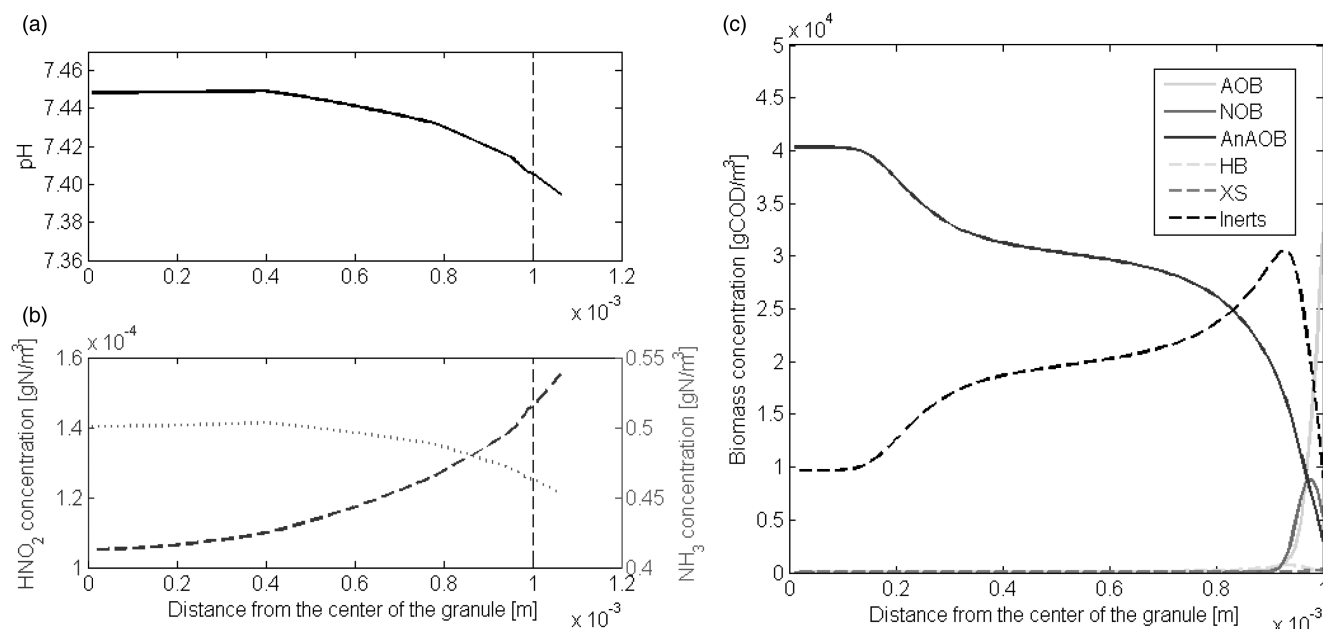


Figure 3 | (a) pH profile with biofilm depth, (b) NH_3 and HNO_2 profiles biofilm with biofilm depth, and (c) biomass composition inside the granular biofilm in scenario 2 simulated with pH effect on microbial growth.

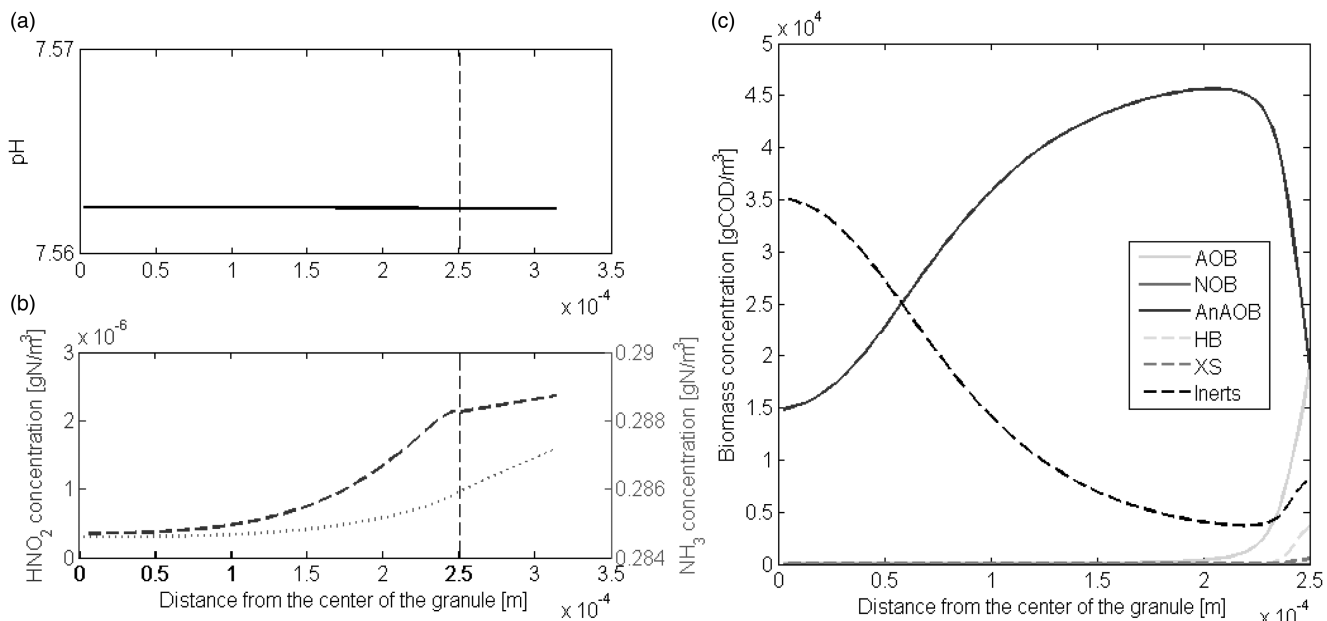


Figure 4 | (a) pH profile with biofilm depth, (b) NH_3 and HNO_2 profiles biofilm with biofilm depth, and (c) biomass composition inside the granular biofilm in scenario 3 simulated with pH effect on microbial growth. The vertical dashed line in (a) and (b) indicates the biofilm/liquid interface.

In the final scenario, the pH profile obtained showed changes in pH over the entire depth of the biofilm (Figure 5(a)). Since a higher load was applied, more substrate was available for both the AOB and the AnAOB. This meant that more TAN was converted, and thus more

protons produced, causing the slope of the pH profile to last the entire depth of the biofilm, similar to scenario 2. The higher load also meant that the AnAOB were present in higher concentrations and dominated the interior of the granules (Figure 5(c)). Despite their increase in amount,

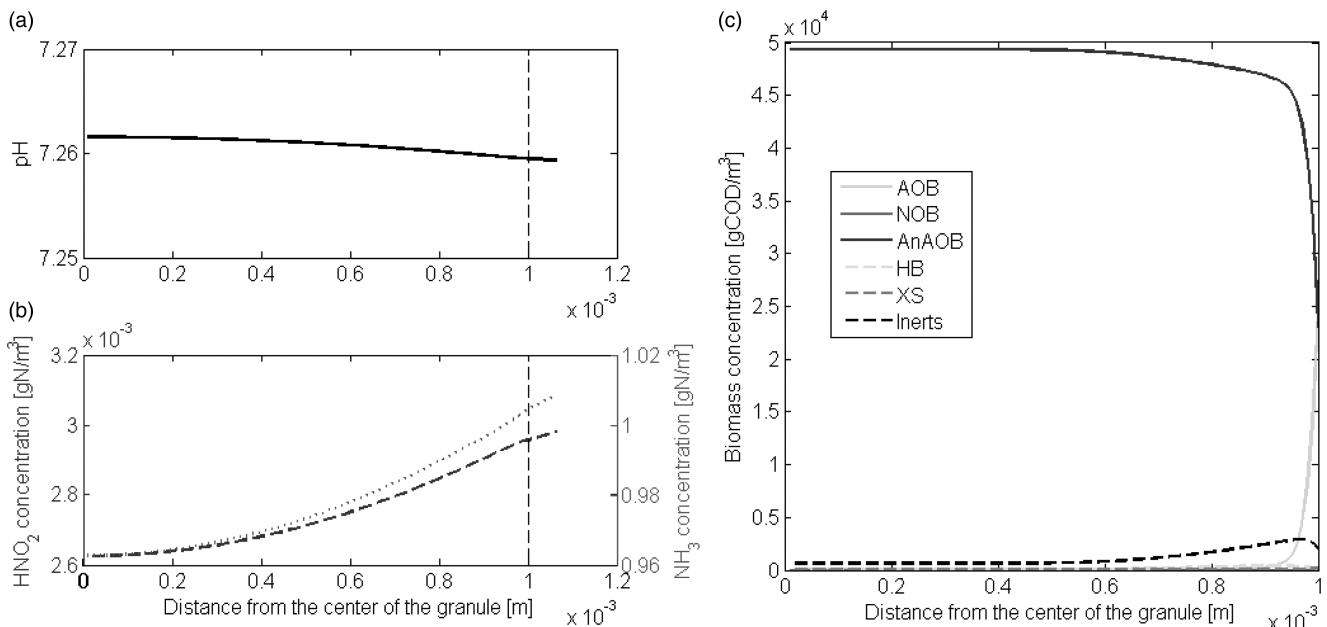


Figure 5 | pH, (a) pH profile with biofilm depth, (b) NH_3 and HNO_2 profiles biofilm with biofilm depth, and (c) biomass composition inside the granular biofilm in scenario 4 simulated with pH effect on microbial growth.

they were not able to convert all the supplied nitrogen, and the overall nitrogen removal efficiency was therefore 75.1% of the supplied nitrogen load.

Despite the difficulty related to determining the appropriate value of the background charge, it is believed that the approach developed and described in this work provides valuable information about how the pH impacts the processes and the relation between microorganisms within the granule. Even though a pH profile could be simulated and constructed over the depth of the biofilm, it was evident that the change in pH over the depth was relatively small (highest in scenario 2 and lowest in scenario 3), due to the buffering capacity of bicarbonate. Thus, the stratification was present for pH, but the difference in impact on the activity potential of the different microbial groups caused by pH change over the depth of the biofilm was relatively small (about 5% at most). Compared to the impact on the activity potential caused by e.g. the stratified oxygen concentration, the impact caused by pH changes on the microbial activity potential was relatively small.

Ultimately, experimental work (e.g. with micro-sensors) should be carried out to give more insight about the actual conditions of pH and background charge inside the granule. Further work also includes investigating the impact of different bicarbonate concentrations and, in relation to this, the effect of CO₂ stripping, especially in systems exposed to high aeration rates.

CONCLUSIONS

A pH simulator was constructed through a numerical solver of a system of nine nonlinear equations and was implemented in the MATLAB software. The pH simulator was coupled to a dynamic granule model by using a look up table to facilitate the computational efficiency. In this way the pH profiles within granular sludge performing the nitrification-anammox process, at a range of different operating points, were simulated. The results showed the following: granules containing proton producing AOB in the outer layers of the granules, next to the biofilm/liquid interface, always resulted in an increasing pH with increasing depth into the biofilm.

A number of approximations were needed to complete the solution of the whole model, e.g. the value of the background charge and bicarbonate concentrations. This was found to have a great impact on the value and shape of the pH profile, and estimating or measuring an appropriate and correct value of these variables is therefore of great

importance. As a conclusion, these results represent a first step in the evaluation of the impact of pH in granular systems. However, more information about the effect on the cell structure and the background charge estimation, supported by experimental essays, will be needed to verify the conclusions and consolidate the pH effect on microbial activities.

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